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Low Power Upconverted Near-IR Light for Efficient Polymeric Nanoparticle Degradation and Cargo Release

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Light-degradable polymers; upconverting nanoparticles; photo-induced degradation; photo-release

Smart polymeric materials, which predictably respond to subtle environmental cues, are some of the most versatile tools in biomedical research, finding applications in tissue engineering, textiles, wound healing, drug delivery, and biosensors.[1] While stable under normal physiological conditions, these polymer matrices can be designed to be sensitive to chemical markers of disease, such as low pH, reactive oxygen species, and specific enzymes,[2] or to external inputs like heat, magnetic field, ultrasound, and light.[3, 4] Light is a very attractive source of energy as it can be applied with a high degree of spatial and temporal resolution. Moreover, with the availability of diverse light sources, including

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Experimental

See Supporting Information for experimental details.

((Supporting Information is available online from Wiley InterScience or from the author)).

M.L.V., M.G., and A.A. designed the experiments, M.G. synthesized the upconverting nanoparticles, N.F. synthesized the light-sensitive polymer, and M.L.V prepared the nanocomposites. M.L.V. performed the experiments and analyzed the data; M.L.V., M.G., and A.A. wrote the paper.

highly tuneable pulsed and continuous wave laser systems, a broad range of parameters (i.e., wavelength, intensity, pulse length, exposure time) can be adjusted precisely to control biomaterial behavior.

UV light-degradable polymers, which respond to photo-cleavage of a protective group by degrading into their component monomers, are known tools for the remote controlled release of therapeutics.[4–6] However, their applications are limited by the short penetration depth of UV light through biological tissue and its detrimental high energy. As a stimulus for minimally invasive therapies in an in vivo setting, the near infrared region (NIR, 750–1000 nm) presents distinct advantages, since its long wavelength allows it to penetrate deep into tissues and its low energy is not harmful to healthy cells.[7] Though very few photo-labile protecting moieties are susceptible to NIR light, some UV-sensitive leaving groups are able to absorb the energy required for photo-cleavage through simultaneous, two-photon absorption.[8, 9] This process, however, requires high peak powers from femtosecond pulsed lasers focused onto a very small cross-sectional area ($1mm^2$ or less) and is generally inefficient and slow due to low two-photon absorption cross sections of the chromophores.[10] Alternatively, incorporating upconverting nanoparticles (UCNPs) has recently emerged as a strategy for harnessing the energy of NIR light to do photochemistry.[11–13] Rather than absorbing two photons simultaneously, UCNPs exploit the physical properties of their lanthanide dopants to sequentially absorb several photons of NIR light and emit a single photon in the UV or visible range.[14] This physical process offers remote access to diverse UV-Vis photochemistry. Incorporating UCNPs could potentially bridge the gap between the available photo-responsive polymers and non-invasive, low-energy NIR light ideal for biomedical applications. We envision using UCNP-assisted photochemistry for a plethora of applications such as remote manipulation of 3D tissue cultures, activation of chemotherapeutics in specific target sites, and delivery of bioactive agents to initiate and study biological phenomena.

Herein, we present the first example of robust depolymerization of a biomaterial triggered by UV upconverted luminescence. As illustrated in Figure 1A, the strategy consists of loading UCNPs, along with bioactive model cargo, in polymeric NPs. Upon excitation at 980 nm, UV photons emitted from the encapsulated $NaYF_4$:Yb.Tm UCNPs are absorbed by the o-nitrobenzyl (ONB) photo-responsive moieties,[9, 15] showing impeccable spectral overlap with the UCNPs' UV emission (Figure 1B). The prevalence of the ONB triggering groups on each cresol monomer ensures efficient degradation through a cascade of cyclization and rearrangement reactions in response to minimal UV exposure (Figure 1C).[4] UCNP-assisted disassembly of micelles and hydrogels has been reported recently,[13] but, to the best of our knowledge, no study demonstrating the ability of UCNP radiative energy to induce degradation of polymer particles and subsequent cargo release has been published to date.

Highly luminescent core-shell $NaYF_4$:Yb.Tm nanoparticles (core = $NaYF_4$: 30% Yb^{3+}, 0.5 % Tm^{3+}; shell = $NaFY_4$) were synthesized following a method published by Carling et al.[11] The core-shell UCNPs possess a uniform rod-like shape and are 33 ± 1 nm in length and 28 ± 1 nm in width, as determined by statistical analysis of the TEM images (Figure S1). These core-shell $NaYF_4$: Yb.Tm crystals display NIR-to-visible and NIR-to-UV

upconversion (Figure 1B) with a sharp excitation maximum at 980 nm (Figure S2). Light-sensitive polymer capsules incorporating consistent concentrations of core-shell UCNPs and a hydrophobic model cargo, coumarin 153 (C153), were obtained using a simple, single-step electrospray process. High voltage was applied to a mixture of polymer, UCNPs, and fluorophores in organic solvent to break the liquid into a jet of very fine aerosol droplets, resulting in a dry powder that can be stored in darkness indefinitely and is easily dispersed in aqueous solutions. Compared to several particle fabrication techniques (e.g. emulsion), electrospray is a commercially viable method of choice. It is fast and simple, requires minimum amounts of solvent, and allows highly efficient encapsulation of both hydrophilic and hydrophobic molecules, as well as inorganic particles and fragile biomolecules.[16] Also, many variables (electrostatic field strength, needle diameter, solution flow rate, concentration) can be tuned to obtain narrow size distributions of nanoscale and microscale particles.[16] Because we did not pursue a particular application for NIR-triggered release, we chose an arbitrary polymeric particle size. We have produced empty and UCNP-containing medium sized particles with diameters varying between 300 and 1000 nm (Figure 2A, B). TEM microphotographs showed good encapsulation efficiency, with no evidence that UCNPs escaped the polymer matrix. The number of UCNPs per NP was tuned by observing loading via TEM imaging, where underloading and overloading could be easily detected by either the presence of multiple empty polymer particles or composite particles falling into pieces with a barely discernible polymer matrix, respectively, and subsequently adjusting the concentration of UCNPs in the electrosprayed solution. Upon irradiation NPs of encapsulating UCNPs at 980 nm, luminescence emanates from the excited UCNPs as blue light, a spectral region where the polymer does not absorb significantly (Figure 2C). Also, efficient radiative energy transfer from the UCNPs to the ONB polymer was observed in the region of spectral overlap (300 – 400 nm) when comparing the luminescence spectrum of free UCNPs in solution with the spectrum of UCNPs trapped in the UV-absorbing polymer matrix (Figure 2D). As expected, luminescence intensity below 400 nm was significantly lower in the spectrum from polymer-entrapped UCNPs, indicating the polymer absorbs most of the light emitted by the UCNPs. To rule out the possibility that the lower UV luminescence collected from the composite polymer material was only a result of polymer-mediated scattering of the UCNPs' luminescence, spectra were also collected from UCNPs dispersed in a mix of $CHCl_3$/CH_3OH (19:1) combined with varying concentrations of light-degradable polymer (Figure S3). While luminescence intensity remained constant above 400 nm, a progressive decrease in the luminescence was detected below 400 nm as the polymer concentration was increased. This again supports the occurrence of efficient energy absorption of the upconverted UV emission by the polymer matrix, which, with adequate excitation energy, should translate into efficient photo-cleavage of the ONB protecting groups and degradation of the polymer carriers.

Degradation of polymer capsules was studied by gel permeation chromatography (GPC). Polymer nanocomposites dispersed in PBS at pH 7.4 were exposed to pulsed laser light (980 nm) for various periods of time (0 – 4 hrs) and different power densities (0.25 – 1 W). After irradiation, degradation was allowed to proceed in PBS (pH 7.4) at 37 °C for 48 hrs. GPC chromatograms (Figure 3A) confirmed successful depolymerization of the UCNP-loaded polymer capsules upon irradiation at 980 nm (1 W), as the peaks decreased in intensity and

shifted to a longer retention time, characteristic of the appearance of degradation products, i.e., monomers, oligomers, polymer building blocks (see GPC chromatograms of expected byproducts, Figure S4). However, no significant change in peak shape was observed when pure polymer NPs were irradiated, indicating that upconverted UV luminescence is required for 980 nm irradiation to induce polymer degradation (Figure 3B). Interestingly, a lack of cytotoxicity of the light-sensitive polymer and the degradation products after light exposure was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. No significant differences in cell viability were also observed between the intact polymer and the degraded polymer (Figure S5). When loaded with UCNPs, the degree of polymer degradation was strongly dependent on irradiation time and laser power density. Average molecular weights extracted from GPC traces (1 W: Figure 3A, 0.5 W: Figure S6) revealed fragmentation directly proportional to irradiation time and laser power density (Figure 3C). UCNP-loaded polymer capsules showed a 60% and a 30% reduction when irradiated for 4 hrs at 1 W and 500 mW, respectively. The degree of degradation and fragmentation of the particles depends strongly upon the extent of ONB group removal, which is greater at high laser intensities and long irradiation time, explaining the difference in changes in polymer molecular weight shown in Figure 3C. After incubation, TEM micrographs confirm degradation of the polymer nanoparticle structure; only then were free UCNPs observed (Figure S7). Attempts to degrade polymer capsules using lower power densities, i.e., 350 and 250 mW, led to unfragmented polymer capsules. Nevertheless, successful depolymerization at 500 mW represents a minimum 6-fold reduction over required laser powers (i.e. > 3 W) previously reported to induce disassembly of polymer carriers using upconverted luminescence.[13] This may be attributed to the nature of the degradable hydrophobic polymer used in this study, which offers the advantage of disassembly on both the nanoscale (disintegration of the nanocarrier) and the molecular scale (polymer fragmentation).[4] Because of this unique polymer backbone fragmentation, fewer triggering events are needed to disassemble the nanocarriers compared to those made of polymers that disassemble by hydrophobicity switch. Although low in intensity, the laser power densities used in this study do not fall within a biologically benign regime.[17] The main limitation is the luminescent materials' weak absorption coefficient (10^{-5} L•g^{-1}•cm^{-1}) and quantum yield (< 0.3%), making high illumination intensities still necessary.[18, 19] However, promising hybrid materials with enhanced upconversion efficiency have been reported recently. By providing either plasmonic coupling or dye-sensitization, upconversion luminescence has been increased by orders of magnitude.[19, 20] These improvements have the potential to transform the upconversion phenomenon into a realistic and viable tool for biological applications.

To evaluate the performance of this new strategy for controlled light-triggered release, polymer capsules containing UCNPs and C153 were dispersed in PBS at pH 7.4 and irradiated at 980 nm with pulsed laser at power varying between 250 to 1000 mW. The release of C153 upon irradiation was followed over time by fluorescence spectroscopy. C153 was chosen to model the release of small molecules from this polymer system because its fluorescence emission maximum reflects the polarity of its surrounding environment. Its well-characterized solvatochromic properties have been widely used to probe heterogeneous environments, such as supramolecular host cavities, micelles, and polymers.[21] The dye

concentration in the particles (~10 mmol per kg of polymer) was adjusted to exceed ~10^{-3} M, so that fluorescence emission from the particle suspension would be self-quenched in the off-state because of non-fluorescent dimers, and would increase following dye release and loss of quenching.[22] Release was proportional to NIR laser power (Figure 4): when excited at 1 W, fluorescence intensity rapidly increased, indicating a fast release of the dye from the polymer capsules into the aqueous environment (Figure 4, open hexagons; see also Figure S8B for emission spectra) that saturated at around 60 min. Irradiation at 500 mW induced similar behavior (Figure 4, solid triangles); however, as expected, less material was released with this lower NIR laser power. C153's emission is very sensitive to solvent polarity, causing a red shift in maximum emission wavelengths from 491 nm to 525 nm upon irradiation with 980 nm light, further indicating release from the hydrophobic environment of the nanoparticles into a more polar medium (Figure S8B). The amplitude of red shift in maximum emission wavelengths is proportional to the amount of dye present in the aqueous medium, so its progression can also be plotted over time to follow the rate of release (Figure S9). In contrast, irradiation at 250 mW caused no change in fluorescence intensity over 90 min (Figure 4, open circles), similar to non-irradiated samples (Figure 4, solid squares; see also Figure S8A for emission spectra). These observations indicate that a minimum amount of energy must be provided to the polymeric material by the UCNPs in order to induce cleavage of the ONB protecting groups, essential for subsequent release from the polymer carrier. The kinetics of release of the dye molecules are faster than those of degradation, suggesting that dye is expelled from the polymer capsules not only because of degradation but also because of the drastic change in hydrophobicity of the particles upon photo-cleavage. Cleavage of ONB from the polymer unmasks a large number of the secondary amino groups and rapidly makes the polymer capsules more permeable to water.[4, 5] Since 980 nm is a water resonant wavelength, an increase of the sample's temperature upon irradiation should be observed as the absorbed photonic energy is transformed into heat, which could trigger a phase change in the polymer capsules and increase their diffusivity enough to release the encapsulated content. To isolate the possible effect of photo-induced heating, we conducted a control experiment in which polymer carriers containing only C153 were exposed to laser irradiation at 1 W for 90 minutes. Upon irradiation, fluorescence intensity increased slightly and the emission maximum wavelength shifted somewhat (Figure S8D, **red circles**; see also Figure S8C for emission spectra). Since irradiation increases sample temperature by 10 °C, this minor dye release can be attributed to a thermally induced phase change in the polymer carrier, which increases diffusivity.[23]

By encapsulating UCNPs in UV light-degradable polymer particles, which respond to photo-cleavage of a protective group by degrading into their component monomers, we could access efficient UV-Vis photochemistry using NIR laser light with great sensitivity. This strategy was used successfully to remotely control the release of a co-entrapped fluorophore payload. While few in vivo examples have been published, therapeutic applications of UCNPs are beginning to emerge,[24] leveraging their NIR sensitivity for photodynamic therapy, release of photo-caged species, and the disruption of UV-sensitive micelles and hydrogels for drug release. The low-power degradation of polymer particles reported herein is the first step to other processing possibilities that could include payload-bearing thin films and other controlled release technologies that rely on bulk polymers.

While we chose not to use biocompatible UCNPs in this study as we did not pursue any biological studies, PEG or silica coatings have been shown to effectively eliminate lanthanide toxicity.[25] By bridging the gap between efficient UV photochemistry and deep-penetrating low energy NIR light, biocompatible formulation of our polymer-UCNP blend materials have the potential to activate previously inaccessible targets both for technical and biological applications with unparalleled spatiotemporal control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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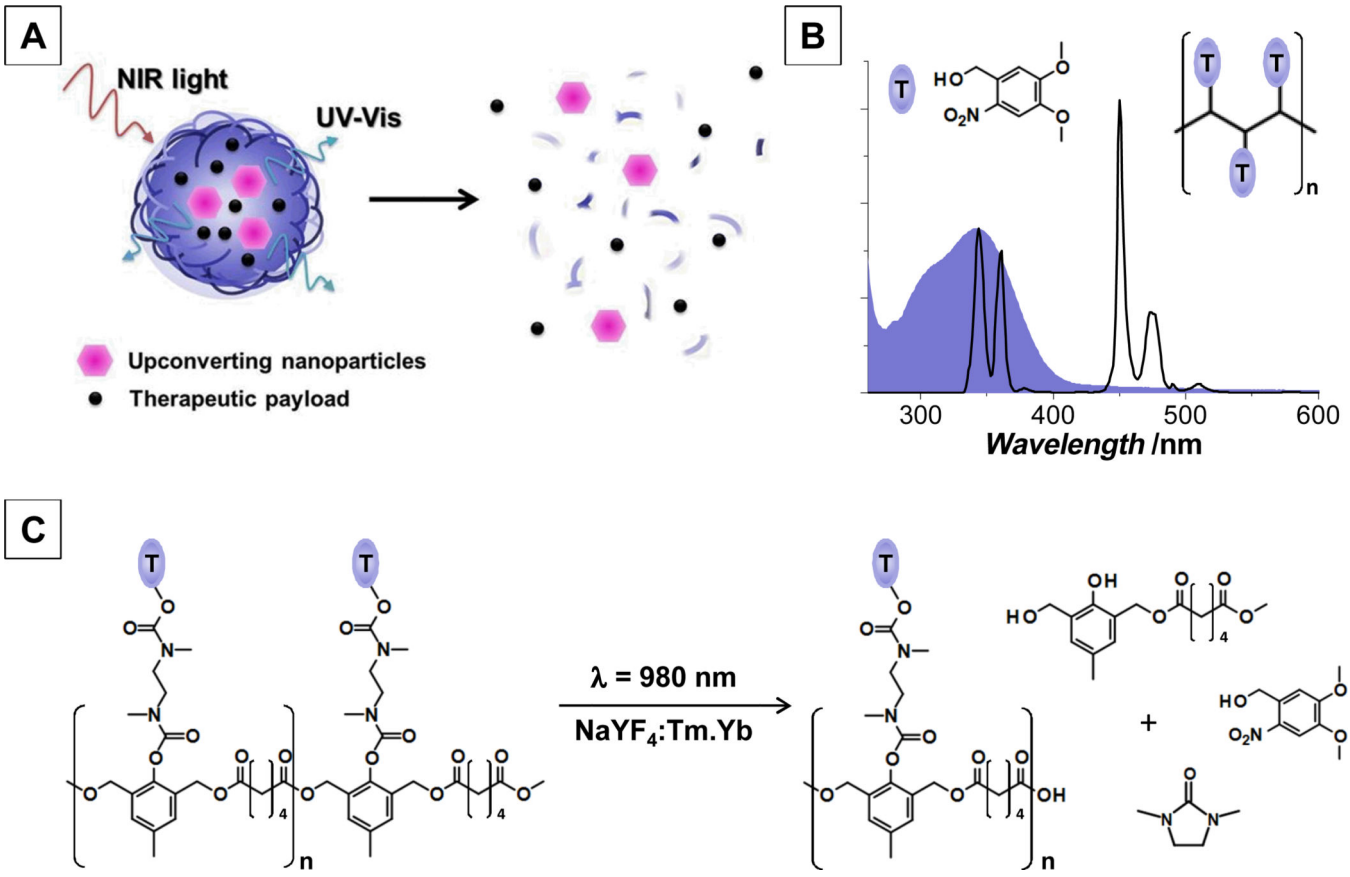

Figure 1.

Schematic representation: (A) upconverted luminescence triggers degradation and release from light sensitive nanoparticles; (B) spectral overlap between the UV emission profile of $NaYF_4$:Yb.Tm core-shell UCNPs (black trace) and the absorption spectrum (shaded blue) of ONB triggering groups; (C) photochemical mechanism of light-triggered degradation.

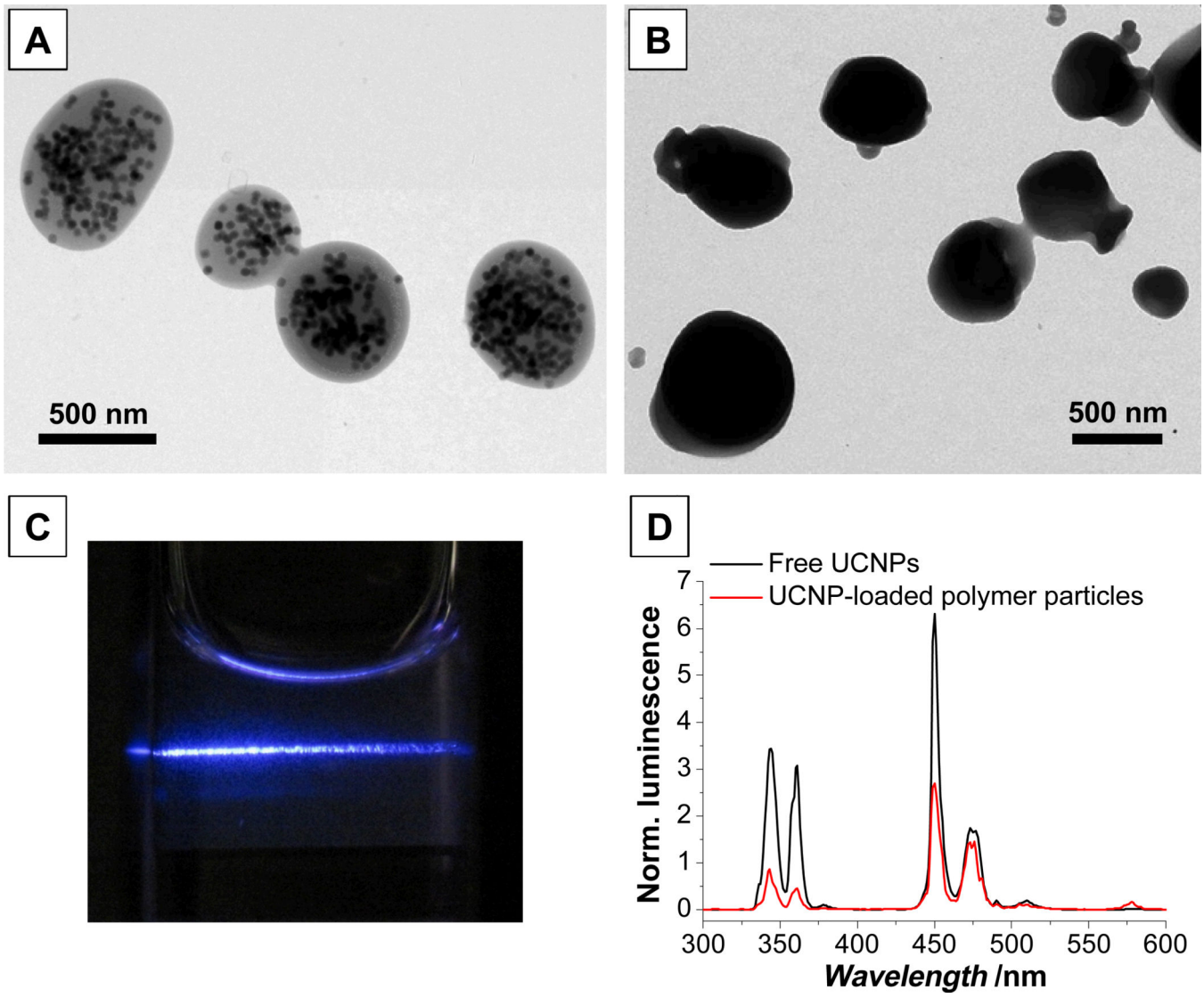

Figure 2.

TEM microphotographs of polycresol NPs (A) with and (B) without UCNPs; (C) Optical photograph of the luminescence emitted from a suspension of UCNP-loaded polymer NPs upon 980 nm laser light exposure; (D) Luminescence spectra of UCNPs free in solution (black line) and trapped in polymer NPs (red line).

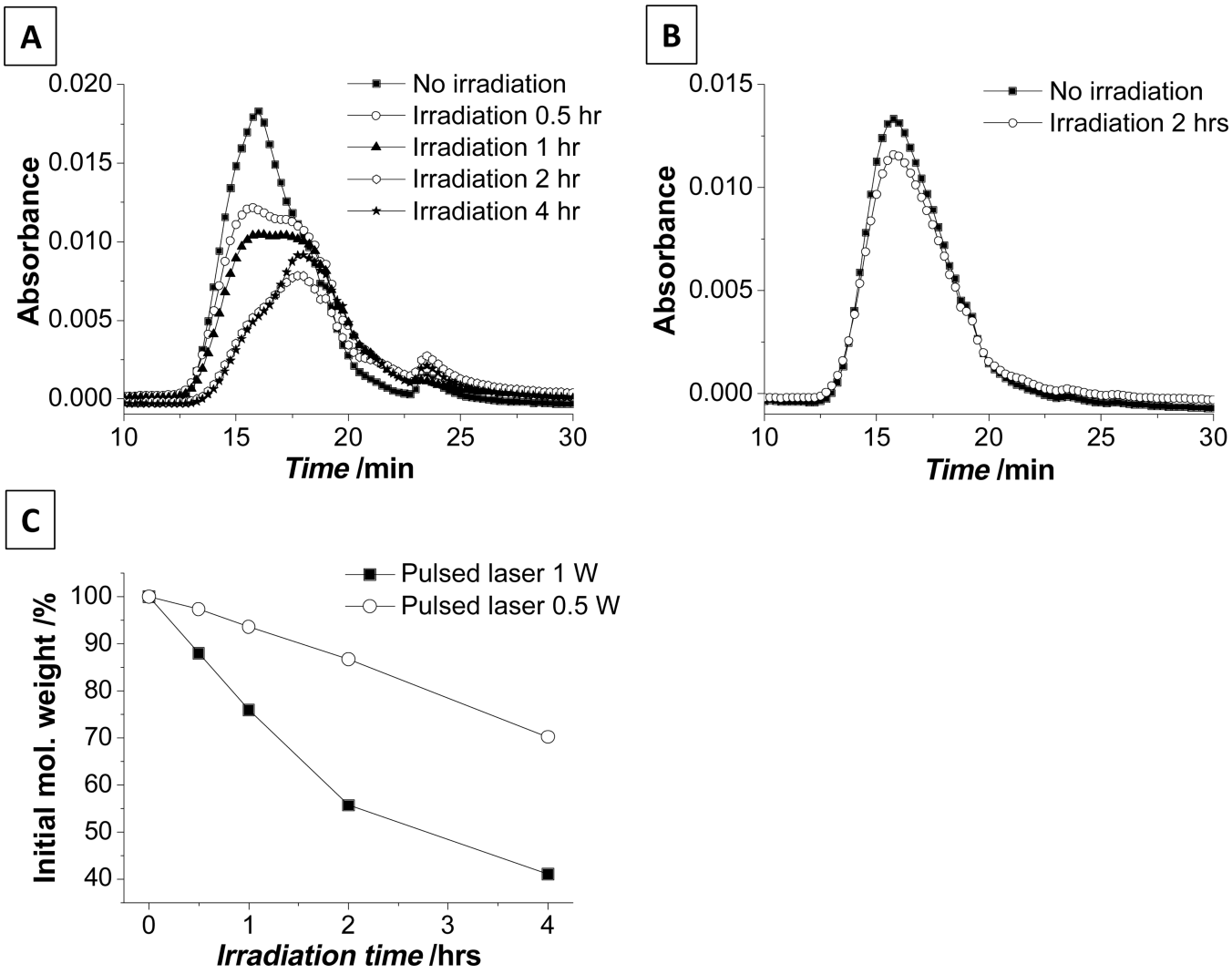

Figure 3.

Upconverted luminescence triggers degradation from light sensitive nanoparticles. GPC chromatograms of (A) UCNP-loaded polymer capsules and (B) polymer capsules without UCNPs, before and after irradiation at 1 W with pulsed laser light (980 nm) for various periods of time; (C) Change in molecular weight of the polymer before and after different irradiation periods at 1 W (solid squares) and 0.5 W (open circles) plotted as the percentage of remaining initial molecular weight. Absorbance measured at 350 nm.

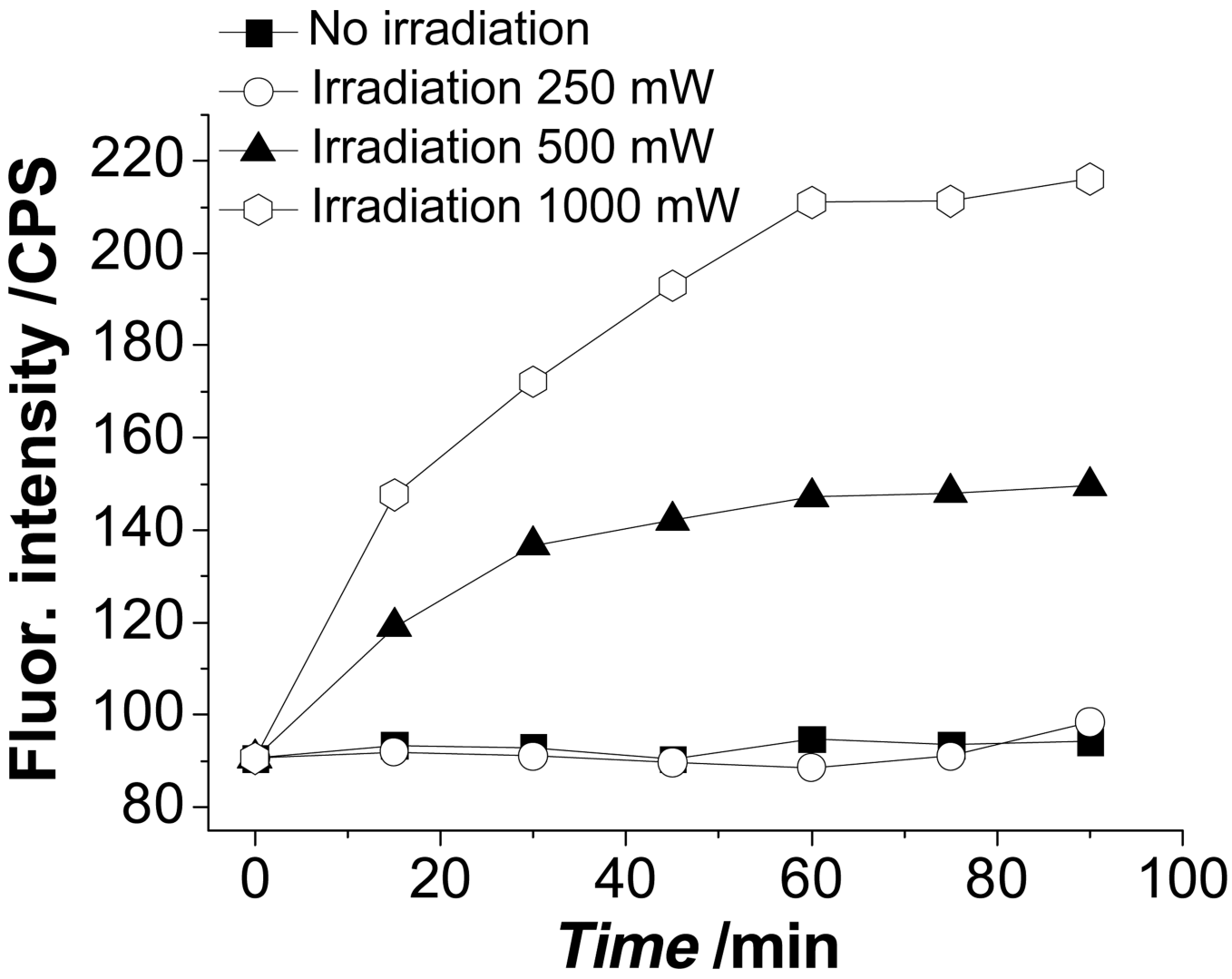

Figure 4.

UCNP-induced release depends strongly on incident laser power. Fluorescence intensity of C153 after 15 min irradiation increments without irradiation (solid squares) and with irradiation (pulsed laser light, 980 nm) at 250 mW (open circles), 500 mW (solid triangles), and 1000 mW (open hexagons).